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BIOSYNTHESIS OF LANDOMYCIN E DEOXYSUGAR PART IN *STREPTOMYCES GLOBISPORUS* 1912: SEQUENCING AND ANALYSIS OF *LNDZ1* AND *LNDZ3* GENES

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DNA fragment of landomycin E biosynthesis gene cluster 1.5 kb in size has been completely sequenced and two open reading frames were identified. Gene *lndZ1* resembles NDP-hexose-3,5-epimerase and *lndZ3* is similar to NDP-hexose-4-ketoreductases. LndZ1 and LndZ3 proteins are suggested to accomplish two last catalytic steps towards deoxysugar L-rhodinose present in landomycin E carbohydrate moiety.

Key words: *Streptomyces*, angucyclines, L-rhodinose biosynthesis, landomycin E.

Many clinically usefull antibiotics contain carbohydrates [4, 13] (fig. 1). Sugars often define the mode of antibiotics action, it's pharmacokinetics and metabolism [4]. There is growing body of data that rational manipulations with carbohydtrate biosynthesis is perspective route towards generation of novel bioactive compounds [5,11].

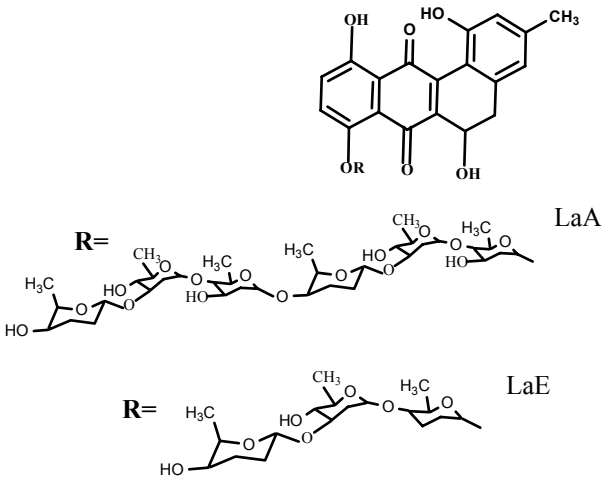

Fig. 1. Structures of landomycins A and E (LaA, LaE) [8]. Polyketide moiety of mentioned landomycins is identical, the difference lies in structure of glycoside chains (R).

Landomycin E (LaE) is angucycline antibiotic whose molecule consists of polyketide framework (landomycinone) modified with trisaccharide D-olivose-D-olivose-L-rhodinose. Landomycin A (LaA) is relative antibiotic where the same deoxysugar motif is repeated twice [2] (fig. 1). Both antibiotics exhibit antitumor and antibacterial activities. Anitumor activity of landomycins elevates as the length of sugar tail increases [8]. These findings prompted us to initiate systematic studies on genes governing LaE deoxysugar biosynthesis. Particularly we are

interested in identification of genes responsible for L-rhodinose formation. Manipulations with these genes will help us to elucidate the substrate specificity of glycosyltransferases involved in LaE production and to generate novel LaE derivatives.

Here we report complete sequencing of two genes *lndZ1* and *lndZ3* which are likely involved in the last steps of L-rhodinose formation. Gene cluster for LaE biosynthesis (*lnd*) in *S. globisporus* 1912 has been partially sequenced and studied through directed mutagenesis [1,6-8, 10]. The cluster for LaE biosynthesis was shown to be widely congruent in it's general architecture with LaA biosynthetic gene cluster (*lan*) from *S. cyanogenus* S136 [10,15] (fig. 2). Downstream of possible antiporter gene *lanJ* 4 genes were identified – *lanZ1*, *lanZ2*, *lanGT3*, *lanZ3* (fig. 2, *A*). Two of them, *lanZ1* and *lanZ3* are supposed to be responsible for biosynthesis of L-rhodinose molecule [15], whereas *lanZ2* and *lanGT3* genes encode possible hexose-synthase and glycosyltrasnferase, respectively, involved in hexasaccharide chain synthesis [14,15]. Respective region of *lnd* cluster was only partially sequenced. Thus we have decided to complete sequencing of this region and compare it's organization with that of *lan* cluster.

Plasmid pUC3.5Pst1 (fig. 2, *B*) was subcloned as *Sma*I and *Sac*I fragments into pUC18 and resulting plasmids were subjected to terminal sequencing from both ends using standard M13p universal and reverse primers. Nucleotide sequence was determined by dideoxynucleotide chain termination method on ALF Express sequencer (Pharmacia). The sequence was analyzed and aligned with the GCG sequence analysis software package (version 8; Genetics Computer Group, Madison, Wis.) and GeneDoc (version 2.6.002). BLAST X searches were performed to find *lndZ1* and *lndZ3* homologues. Conserved domain search was done with CDD

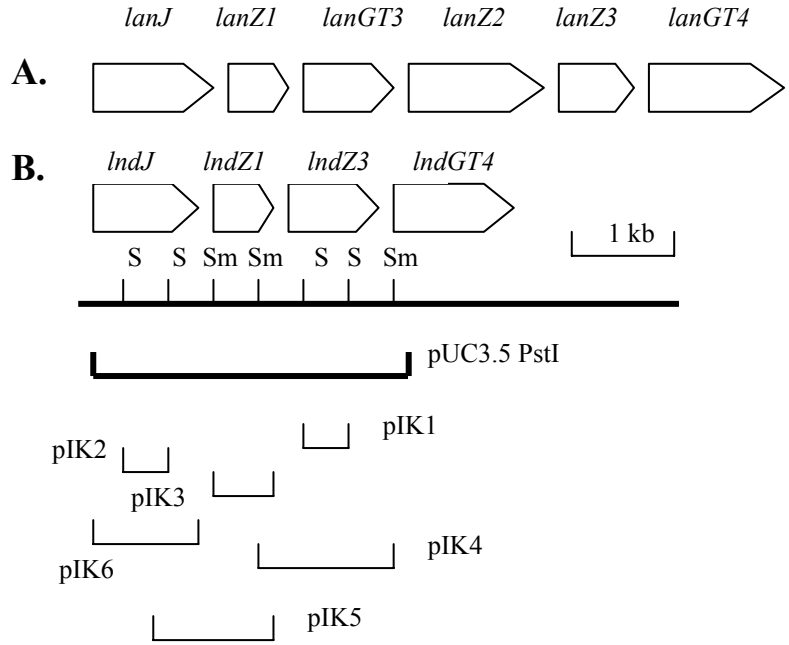

Fig. 2. A. Organization of fragment of *S. cyanogenus* S136 *lan* cluster. Gene functions are given in [15]. B. Genetic and restriction map of fragment of *S. globisporus* 1912 *lnd* cluster. Gene functions – see [1]. Below subclones are shown used for sequencing. Abbreviations: S – *Sac*I, Sm – *Sma*I.

engine (NCBI server) and phylogenetic trees were built using DNASTAR software (CLUSTAL W algorithms). GenBank accession numbers to *lndZ1* and *lndZ3* sequences are AY608714 and AY608715, respectively.

Gene *lndZ1* begins with ATG which is preceded by putative RBS (GAGG) 5 nt upstream of *lndZ1*. First in-frame stop codon (TGA) is recognized 570 bp downstream of start codon, thus specifying 190 aa protein. Gene *lndZ3* starts from unusual valine codon (GTG). It is separated by 8 nt sequence from *lndZ1* stop codon. Putative RBS for *lndZ3* is identified 34 nt uspream of start codon, thus lying in *lndZ1* coding sequence. First in-frame stop codon (TGA) lies 927 nt downstream of *lndZ3* start codon defining 309 aa protein. Sequencing was performed beyond *lndZ3* stop codon up to the 5'-end of *lndGT4* gene [8]. 57 bp intergenic sequence separates *lndZ3* and *lndGT4*. No nucleotide motifs typical for *Streptomyces* promoters [12] could be identified within this region. Taking into account these data and previous studies on *lndGT4lndZ4lndZ5* genes [8], we suggest tentatively that genes *lndZ1lndZ3lndGT4lndZ4lndZ5* form single transcriptional unit. At this moment we have no sequences upstream of *lndZ1* long enough to perform promoter searches. It is possible that *lndJ* transporter gene also falls into this transcriptional unit. Further sequencing will clarify this question. Genes *lndZ1* and *lndZ3* have high GC content (68 and 74%, respectively) with strong bias towards GC usage in third codon position, typical for streptomycete genes [12].

Probable product of *lndZ1* translation shows 85% of identity and 91% of similarity to putative NDP-hexose-3,5-epimerase LanZ1 from *S.cyanogenus* S136 *lan* cluster [15], 61% and 69% to rhamnose-3,5-epimerase Gra-orf25 from *S.violaceoruber* granaticin biosynthesis gene cluster (GenBank accession number - CAA09646), 49 and 58% to possible epimerase UrdZ1 from urdamycin producing *S.fradiae* [3]. Putative LndZ3 exhibits 62% of identity and 72% of similarity to putative NDP-hexose-4-ketoreductase LanZ3 from *lan* cluster [15], 46% and 58% to hexose-ketoreductase UrdZ3 from urdamycin cluster [3], 45 and 53% to NanG4, presumed hexose-4-ketoreductase involved in biosynthesis of polyether nanchangmycin by *S. nanchangensis* (AAP42863), 40 and 49% to diphospho-4-keto-2,3,6-trideoxyhexulosoreductase DnmV from daunorubicin gene cluster of *S.peucetius* (AAB63047), 36 and 46% to hexose-4-ketoreductase SnoG from nogalamycin producer *S.nogalater* (AAF01816). In general all BLAST hits to both sequenced genes fall into single group of enzymes catalyzing last steps of deoxysugar formation. CDD searches and phylogenetic comparisons (fig. 3, *A*, *B*) also indicate that LndZ1 is NDP-hexose-3,4-epimerase and LndZ3 is NDP-hexose-4-ketoreductase. These enzymes are suggested to be involved in two last sequential steps of L-rhodinose formation (fig. 4). Further gene knockout experiments will be performed to substantiate this speculation.

Genes *lndZ1* and *lndZ3* are separated by 8 nt spacer (fig. 5), whereas in *lan* cluster two additional genes *lanZ2* and *lanGT3* are localized between *lanZ1, lanZ3* [15]. Detailed comparative analysis of *lan* and *lnd* nucleotide sequences allowed us to speculate, that there was deletion within *lnd* cluster 2806 bp in length (comprising entire *orfs* for *lanZ2* and *lanGT3* homologues) which was substituted with insertion of 401 bp sequence restoring correct in-frame stop codon of *lndZ1* and start codon of *lndZ3*. Comparison of LndZ1 and LndZ3 putative aminoacid sequences shows that 7 residues of LanZ1 (SEWPGGN) are substituted in LndZ1 with APCAA. LndZ3 starts with valine V, which corresponds to 10^{th} aminoacid of LanZ3. Thus first nine aminoacids (MTGTGGQRR) found in LanZ3 are absent in LndZ3. Interestingly that UrdZ3 from urdamycin gene biosynthetic cluster of *S.fradiae* has the same truncated N-terminus as LndZ3 has [3]. The data presented indicate that deletion occurred in evolutionary history of *S. globis-*

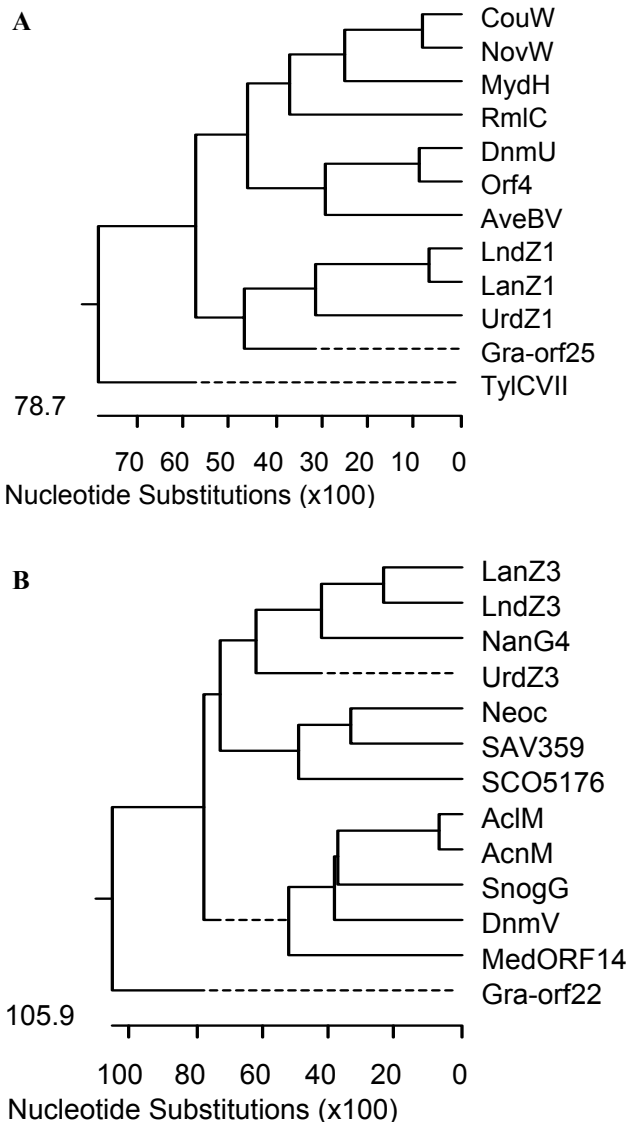

Fig. 3. Phylogenetic trees showing the relatedness of different hexose-epimerases to probable LndZ1 protein (**A**) and hexose-reductases – to LndZ3 (**B**). Functions of LanZ1, UrdZ1, TylCVII, Gra-orf25, LanZ3, UrdZ3, NanG4, DnmV, SnoG are given above in the text. Other proteins: CouW – involved in coumermycin biosynthesis in *S. rishiriensis,* NovW – novobiocin production in *S. spheroides*, MydH - macrolide mycinamicin biosynthesis in *Micromonospora griseorubida,* RmlC – deoxysugar biosynthesis in *B. tuberculosis*, DnmU – daunorubicin production in *S.peucetius*, Orf4 – anthracycline biosynthesis in *S. griseus*, AveBV – deoxysugar biosynthesis in *S.avermitilis*, Neoc - enediyne neocarzinostatin production by *S.carzinostaticus*, SAV359 - putative hexose-reductase from *S.avermitilis*, SCO5176 - putative hexose-reductase from *S.coelicolor* , AclM, AcnM – sugar biosynthesis during aclacinomycin production in *S.galilaeus*, MedORF14 – medermycin biosynthesic in *S. sp.* AM-7161 , Gra-orf22 – granaticin biosynthesis in *S.violaceoruber*.

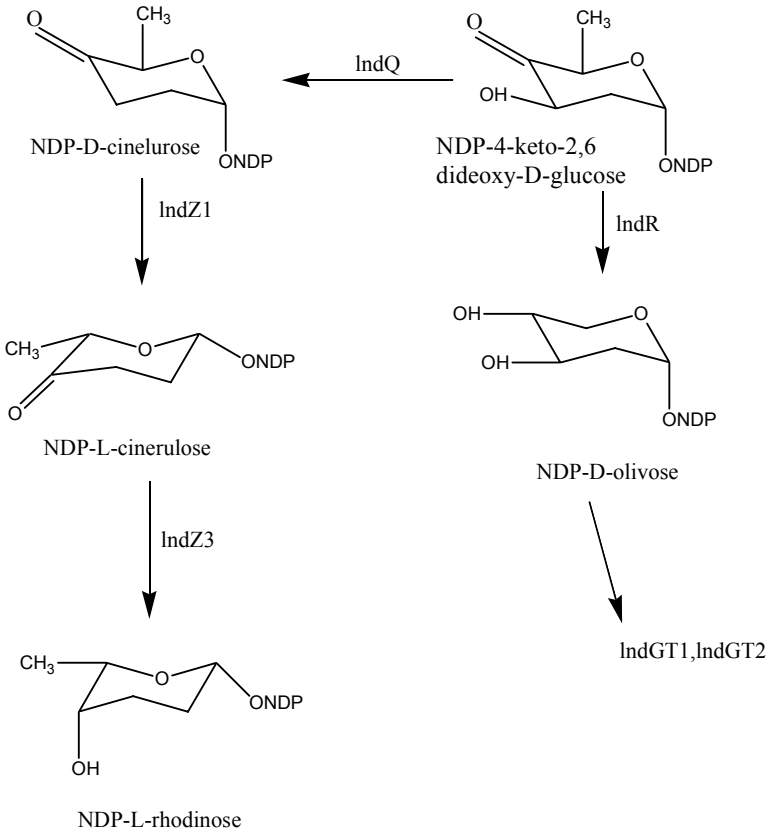

Fig. 4. Putative sequence of reactions during L-rhodinose and D-olivose biosynthesis from common precursor NDP-4-keto-2,6-dideoxy-D-glucose.

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                                                    stop lndZ1
                *       540         *       560         *       580
con : CCTCGCTGGCGGCCACCCTCGCCTCCGGAGTCCTGGCTCCGTGCGCCGCCTGACCGGCACCCGG :   585

          start lndZ3

              *       600         *       620         *       640         *
con : GTGGTGGTCCTGGGCGGCAGCGGCTTCCTCGGCCGGCACCTGCGCACCGCCTACCGGCGTCCGGG :  650
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Fig. 5. Fragment of *lndZ1* and *lndZ3* sequences with intergenic region.

porus 1912 *lnd* cluster leading to removal of *lanZ2* and *lanGT3* homologues. Latter genes are thought to be responsible for LaE conversion into LaA molecule in *S.cyanogenus* S136 [14]. Therefore, deletion of *lanZ2* and *lanGT3* counterparts from *lnd* cluster accounts for LaE production phenotype of *S. globisporus* 1912.

Sequencing of *lndZ1* and *lndZ3* genes set first stage for detailed investigations on biosynthesis of LaE deoxysugar moieties We also revealed differences in genetic organization of *lnd* and *lan* clusters which correlate with differences in LaE and LaA structures.

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БІОСИНТЕЗ ДЕЗОКСИЦУКРОВОЇ ЧАСТИНИ ЛАНДОМІЦИНУ Е В *STREPTOMYCES GLOBISPORUS* 1912: СЕКВЕНУВАННЯ ТА АНАЛІЗ ГЕНІВ *LNDZ1* ТА *LNDZ3*

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Визначено нуклеотидну послідовність фрагмента *lnd*-кластера *S. globisporus* 1912 розміром 1,5 т.п.н. та виявлено дві відкриті рамки зчитування. Ймовірним продуктом гена *lndZ1* є NDP-гексозо-3,4-епімераза, а *lndZ3* - NDP-гексозо-4-кеторедуктаза. Припускають, що кодовані ними білки задіяні у каталізі двох останніх етапів формування дезоксицукру L-родинози, яка є в складі молекули ландоміцину Е.

Ключові слова: *Streptomyces*, ангуцикліни, біосинтез L-родинози, ландоміцин Е.

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